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SPAD-502 and atLEAF CHL PLUS values provide good estimation of the chlorophyll content for *Hevea brasiliensis* Müll. Arg. Leaves

Nilai SPAD-502 dan atLEAF CHL PLUS menghasilkan perkiraan kandungan klorofil daun Hevea brasiliensis *Müll. Arg. yang akurat*

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Abstrak

Pengukuran kandungan klorofil menggunakan metode destruktif relatif tidak efisien dalam hal jumlah sampel, biaya, dan waktu yang diperlukan. Untuk mengatasi hal ini, estimasi kandungan klorofil daun dapat dilakukan dengan metode non-destruktif menggunakan klorofil meter. Penelitian ini bertujuan untuk mendapatkan persamaan untuk mengkonversi nilai SPAD-502 dan atLEAF CHL PLUS (indikator kandungan klorofil relatif) menjadi nilai perkiraan kandungan klorofil daun karet (mutlak). Sebanyak 20 contoh daun karet klon SP 217, PB 260, dan GT1 diukur tingkat kehijauan daunnya menggunakan SPAD-502 dan atLEAF CHL PLUS. Sampel daun tersebut kemudian diukur kandungan klorofilnya menggunakan prosedur standar laboratorium. Analisis regresi dan korelasi dilakukan dengan menggunakan perangkat lunak SAS v.9. Hasil analisis menunjukkan bahwa nilai SPAD-502 dan atLEAF CHL PLUS mempunyai korelasi yang tinggi, sehingga kedua alat tersebut dapat saling menggantikan satu sama lain untuk memperkirakan kandungan klorofil daun karet. Selain itu, nilai yang dihasilkan oleh kedua alat klorofil meter tersebut dan nilai kandungan klorofil mutlak yang dihasilkan dari analisis laboratorium pada klon karet SP 217, PB 260, GT1, dan gabungan semua klon menunjukkan hubungan yang erat dengan nilai Koefisien Determinasi (R^2) yang tinggi serta Galat Akar Rerata Kuadrat (GARK) dan Koefisien Keragaman (KK) yang rendah. Oleh karena itu, dengan mempergunakan persamaan yang dihasilkan dari penelitian ini, kedua alat klorofil meter tersebut dapat digunakan untuk memprediksi kandungan klorofil daun karet secara akurat, cepat, dan tidak merusak sampel daun.

[Kata kunci: atLEAF CHL PLUS; Hevea brasiliensis*; kandungan klorofil; persamaan konversi; SPAD-502]*

Abstract

Measurement of chlorophyll content using destructive methods is not efficient due to a large number of samples, cost, and time needed. Estimation of chlorophyll content by nondestructive methods using handheld chlorophyll meter may be considered to improve efficiency. This research aimed to determine the formula to convert SPAD-502 and atLEAF CHL PLUS values (relative indicator of chlorophyll content) to estimated (absolute) rubber leaves chlorophyll content. Twenty leaves of rubber plant were measured using SPAD-502 and atLEAF CHL PLUS at the same time to determine SPAD-502 and atLEAF CHL PLUS values. The measured leaves were then collected to determine the chlorophyll content using a standard laboratory procedure. Regression and correlation analyses (among 3 methods) were conducted using SAS v.9 software. The results showed that between SPAD-502 and atLEAF CHL PLUS values were closely correlated, hence both of the devices can substitute each other to estimate rubber leaf chlorophyll content. In addition, the relationship between atLEAF CHL PLUS and SPAD-502 values with actual chlorophyll content of rubber clone SP 217, PB 260, GT1, and all clones (general) were significant with high coefficient of determination (R^2) as well as low Root Mean Square Error (RMSE) and Coefficient of Variation (CV). Therefore, by using formula determined in this study, both atLEAF CHL PLUS and SPAD-502 can be suggested for accurate, fast, and non-destructive estimation of chlorophyll content of rubber plant leaf.

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[Keywords: atLEAF CHL PLUS, chlorophyll content, conversion formula, *Hevea brasiliensis*, SPAD-502]

Introduction

Chlorophyll is specialized light-absorbing green pigment that play an essential role in capturing light energy for photosynthesis processes (Limantara *et al.*, 2015; Taiz & Zeiger, 2002). Low chlorophyll content can directly limit the potential rate of photosynthetic process and hence limit crop production (Curran *et al.*, 1995; Filella *et al.*, 1995; Richardson *et al.*, 2002). In addition, chlorophyll content of leaves has a close relationship on crop yield both in normal and drought stress condition (Guo *et al.*, 2008). Furthermore, the ratio of chlorophyll and carotenoids could be a good indicator of stress in plants (Hendry & Price, 1993; Netto *et al.*, 2005). Therefore, chlorophyll content is commonly used as a variable to figure out the condition of plants especially for drought-tolerant genotype screening (da Silva *et al.,* 2012).

For screening purpose, measurement of chlorophyll using destructive methods (laboratory analysis) is not efficient due to a large number of samples required. To cope with this problem, measurement of chlorophyll content can be conducted by nondestructive methods using handheld chlorophyll meter. Recently, there are some handheld chlorophyll meter commonly used to measure chlorophyll content of higher plant leaf based on the light absorbance and or reflectance by intact leaves (Richardson *et al*., 2002), for example SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan) and AtLEAF CHL PLUS chlorophyll meter (FT Green LLC, Willmington, DE). These devices provide simple, quick, and inexpensive measurement of chlorophyll content (Coste *et al*., 2010; Ruiz-Espinoza *et al.,* 2010; Zhu *et al.,* 2012). Measurement of chlorophyll content based on absorbance and or reflectance of light by leaf generally generates an index value not an absolute chlorophyll content per leaf area unit or per leaf tissue weight (Ruiz-Espinoza *et al*., 2010).

SPAD-502 detects the index value of the leaves by sequential measurement of transmittance of red (650 nm) and infrared (940 nm) light using photodiode detectors (Kapotis *et al*., 2003; Minolta, 1989; Uddling *et al*., 2007). The 650 nm wavelength is the spectral region of light that associated with peak activity of chlorophyll, while the 940 nm wavelength is needed for the SPAD calibration as well as leaf thickness and water content compensation (Hawkins *et al*., 2007). Similar to SPAD-502, atLEAF CHL PLUS chlorophyll meter also uses two wavelengths (640 and 940 nm) of light-emitting diodes that being transmitted through the leaf (FT GREEN LLC, 2019). The obtained values of both devices should be calibrated using actual chlorophyll content (can be determined by using standard laboratory spectrophotometric methods) as the control to determine the absolute chlorophyll content of certain plant species (Kapotis *et al*., 2003).

A study to compare chlorophyll values generated from SPAD-502 and atLEAF CHL PLUS has been conducted on five plant species, namely canola, wheat, barley, potato, and corn. This study concluded that the values of relative chlorophyll content generated from both devices were strongly correlated and these devices can substitute one to another (Zhu *et al*., 2012). Furthermore, some research also confirmed the high degree of correlation between chlorophyll meter index value (relative indicator of chlorophyll content) and absolute chlorophyll content as well as deduced the various equations to convert chlorophyll meter value to estimated (absolute) chlorophyll content (Coste *et al*., 2010; Hawkins *et al*., 2009; Kapotis *et al*., 2003; Markwell *et al*., 1995; Novichonok *et al*., 2016; Richardson *et al*., 2002; Ruiz-Espinoza *et al*., 2010; Steele *et al*., 2008; Uddling *et al*., 2007; van den Berg & Perkins, 2004; Zhu *et al*., 2012). Many equations to convert chlorophyll meter index value to estimated chlorophyll content for many plant species are available, but the equation for rubber tree (*Hevea brasiliensis* Müll. Arg.) is not available yet. Therefore, this research was aimed to deduce the equation to convert the index values generated from SPAD-502 and atLEAF CHL PLUS to estimated (absolute) chlorophyll content.

Materials and Methods

This research was conducted at Plant Science Laboratory, Faculty of Agriculture, Gadjah Mada University, Yogyakarta in October 2019. Rubber leaf samples (clone SP 217, PB 260, and GT1) were taken from one whorl rubber planting material. These planting materials were originated from Indonesian Rubber Research Institute in Sembawa, Banyuasin, South Sumatra. The planting materials were brought and maintained in Yogyakarta until new leaf whorl had been formed. Leaves sampling has been conducted three months after the first leaf formation.

To compare SPAD-502 and atLEAF CHL PLUS index values with actual chlorophyll content determined by standard laboratory procedures, 20 leaves of rubber plants (*Hevea brasiliensis* Müll. Arg.) were measured using SPAD-502 and atLEAF CHL PLUS at the same time on October 09, 2019. The leaf samples were taken from two to three month old of SP 217, PB 260, and GT1 rubber plant clones. The plants with various ages were chosen to get various chlorophyll contents of rubber leaves. The measured leaves were then collected to determine the actual chlorophyll content using a standard laboratory procedure. Determination of actual chlorophyll content of the leaves were conducted at Plant Science Laboratory, Faculty of Agriculture, Gadjah Mada University using destructive method (Arnon, 1949).

Prior to the measurement of leaves greenness using SPAD-502 and atLEAF CHL PLUS chlorophyll meter, leaf surfaces were cleaned from dust and other dirt. On each rubber leaf (trifoliate leaf), three SPAD-502 and atLEAF CHL PLUS readings were taken sequentially for 20 rubber leaves. For calculation of correlation between SPAD-502 and atLEAF CHL PLUS values, 60 pairs of observation values were used. Furthermore for deduction of formula to convert SPAD-502 and atLEAF CHL PLUS values to absolute chlorophyll content, the three readings per leaf were averaged to generate a single values per leaf. Before using SPAD-502 chlorophyll meter, this device should be calibrated by pressing measure button without any sample in the sample slot. Furthermore, atLEAF CHL PLUS had been calibrated by the manufacturer and can be used directly by pressing the measure button. The measurement was conducted by locating SPAD-502 and atLEAF CHL PLUS sensors on the leaf lamina and pressing the measure button. The leaf lamina should be fully covered by the sensor, hence the interferences of leaf veins and midribs were avoided.

For chlorophyll content measurement, one gram of rubber leaf sample was ground in a mortar and diluted using 20 mL of 80% acetone. The grinded leaf tissue was then filtered. The absorbance of the filtrate was measured on 645 and 663 nm wavelengths. In addition, pure acetone was used for the blank. Chlorophyll *a*, *b*, and total chlorophyll content (Chlorophyll *a* + Chlorophyll *b*) were determined by using the following equations (Arnon, 1949) :

$$Chlorophyll\ a = \frac{12.7\ (A663) - 2.69\ (A645)\ x\ V}{1000\ x\ W}\ mg/g$$

$$Chlorophyll\ b = \frac{22.9\ (A645) - 4.68\ (A663)\ x\ V}{1000\ x\ W}\ mg/g$$

$$Total\ chlorophyll = \frac{20.2\ (A645) + 8.02\ (A663)\ x\ V}{1000\ x\ W}\ mg/g$$

Where, A = absorbance at specific wavelength
V = final volume of chlorophyll extract in 80% acetone
W = fresh weight of tissue extracted

Regression and correlation analyses between SPAD-502 and atLEAF CHL PLUS values as well as actual chlorophyll content were conducted using proc reg and proc corr of SAS v.9 (SAS Institute Inc., 2002).

Results and Discussion

Both SPAD-502 and atLEAF CHL PLUS did not produce an absolute estimation of chlorophyll content data, whether it is chlorophyll a, b, or total chlorophyll. They produced an index value that should be converted to produce estimation of chlorophyll content of certain plant species. For rubber leaves, the relationships between SPAD-502 and atLEAF CHL PLUS values with actual chlorophyll content are presented in Figure 1, Table 1 and Table 2, respectively.

Figure 1 shows that regression of SPAD-502 and atLEAF CHL PLUS values, respectively, with actual chlorophyll content of rubber leaves were linear with a varied slope between clones and chlorophyll types. The linear relationship between the relative indicator of chlorophyll content and the actual (absolute) chlorophyll content was also reported by other authors (Kapotis *et al*., 2003; Mendoza-Tafolla *et al*., 2019; Ruiz-Espinoza *et al*., 2010; Zhu *et al*., 2012). This evidence was different with Coste *et al*. (2010) and Dong *et al*. (2019) that found that the sensitivity of SPAD decreases with the increasing chlorophyll content.

Among the clones, SP 217 had the highest slope and PB 260 had the lowest slope. Based on the chlorophyll types, total chlorophyll (a+b) had the highest slope and chlorophyll b had the lowest slope. The differences in the regression formula among rubber clones may be influenced by differences in leaf characteristics especially leaf thickness (specific leaf weight) (Gomez & Hamzah, 1980; Martins & Zieri, 2003). The previous experiment observed that specific leaf weight affected the chlorophyll content (a, b, and a+b) of leaves (Ruiz-Espinoza *et al*., 2010), hence different rubber clones have different regression formula. Furthermore, the relationship between atLEAF CHL PLUS and SPAD-502 values with actual chlorophyll content of clone SP 217, PB 260, and GT1 were significant with high coefficient of determination (R^2) (Table 1 and 2).

Table 1 and 2 showed that RMSE and CV of the regression analysis for all clones were low, that indicated the error was small and the estimated chlorophyll a, b, and a+b (total chlorophyll) contents were similar to the observed (actual) chlorophyll a, b, and a+b (total chlorophyll) content respectively. Therefore, both atLEAF CHL PLUS and SPAD-502 can be used to make a fast prediction of chlorophyll content of rubber plant leaves including chlorophyll a, b, and a+b (total chlorophyll).

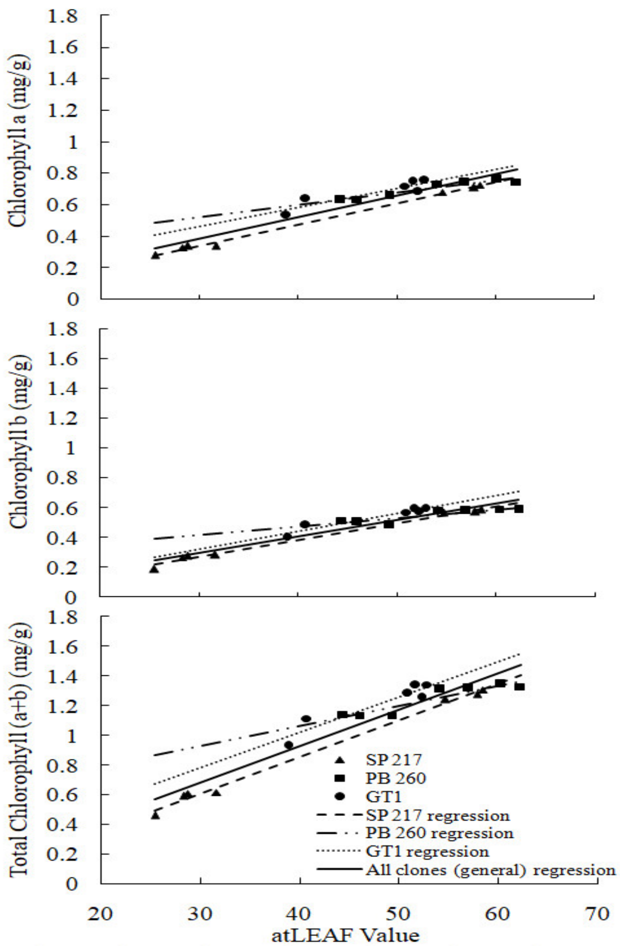

Figure 1. Regression between SPAD-502 and atLEAF CHL PLUS values and actual chlorophyll content of three rubber clones.

Gambar 1. Regresi antara nilai indeks SPAD-502 dan atLEAF CHL PLUS dengan kandungan klorofil aktual pada tiga klon karet.

Figure 1, Table 1 and 2 also showed that the all regression line equations had a positive slope, varied from 0.005 to 0.024 for atLEAF CHL PLUS and 0.009 to 0.021 for SPAD-502. It means that more green leaf colour (the more value of SPAD-502 and atLEAF CHL PLUS values) indicated the higher actual chlorophyll content. From all clones, PB 260 had the lowest slopes among clones. For SPAD-502 observation, clone PB 260 regression lines intersected with general clone regression line at about 40.5 SPAD-502 value. Whereas for atLEAF CHL PLUS observation, clone PB 260 regression lines intersected with general clone regression line at about 50 atLEAF CHL PLUS value. It indicated that for below 40.5 SPAD-502 value and 50 atLEAF CHL PLUS value, at the same index value, actual chlorophyll content of PB 260 was higher than other clones and vice versa. It also indicated that at the same actual chlorophyll a, b, and a+b (total chlorophyll) content (at SPAD-502 value below 40.5 and atLEAF CHL PLUS value below 50), the leaf of PB 260 clone was less green than other clones and vice versa. In general, to estimate absolute chlorophyll a, b, and a+b (total chlorophyll) content from leaf greenness observed by SPAD-502 and atLEAF CHL PLUS, we can use the equations in Table 1 and 2, respectively.

In this research, we also found that the results of leaf grenness observation using SPAD-502 and atLEAF CHL PLUS had a close relationship. Regression and correlation analyses on paired chlorophyll index data of 60 rubber leaf samples observed using SPAD-502 and atLEAF CHL PLUS showed that both devices produced relative indicator of chlorophyll content values that highly correlated each other with a coefficient of determination (R^2)>0.85 (Figure 2).

Table 1. Regression equation to convert SPAD-502 values (x) to estimated rubber leaf chlorophyll content (mg/g) (y)
Tabel 1. Persamaan regresi untuk mengkonversi nilai indeks SPAD-502 (x) menjadi perkiraan kandungan klorofil daun karet (mg/g) (y)

Variable *Variabel*		Regression *Regresi*	R^2 *R^2*	RMSE *GARK*	CV *KK*
Clones *Klon*	Chlorophyll *Klorofil*				
SP 217	Chlorophyll a	y = 0.0117x + 0.1247	0.9981	0.00984	2.03630
	Chlorophyll b	y = 0.0099x + 0.0865	0.9874	0.02155	5.53632
	Chlorophyll a + b	y = 0.0216x + 0.2114	0.9956	0.02773	3.17976
PB 260	Chlorophyll a	y = 0.0055x + 0.4498	0.9026	0.01939	2.78029
	Chlorophyll b	y = 0.0040x + 0.3673	0.7306	0.02608	4.76341
	Chlorophyll a + b	y = 0.0096x + 0.8158	0.8516	0.04263	3.42566
GT1	Chlorophyll a	y = 0.0107x + 0.2850	0.8381	0.03776	5.57906
	Chlorophyll b	y = 0.0100x + 0.1660	0.8684	0.03143	5.88344
	Chlorophyll a + b	y = 0.0207x + 0.4501	0.8653	0.06579	5.43400
All clones (general)	Chlorophyll a	y = 0.0112x + 0.1974	0.8465	0.06549	10.62880
	Chlorophyll b	y = 0.0091x + 0.1476	0.8447	0.05362	10.98465
	Chlorophyll a + b	y = 0.0203x + 0.3451	0.8500	0.11708	10.60573

R^2: Coefficient of Determination. RMSE: Root Mean Square Error, CV: Coefficient of Variation.
R^2: Koefisien Determinasi, GARK: Galat Akar Rerata Kuadrat, KK: Koefisien Keragaman

Table 2. Regression equation to convert atLEAF CHL PLUS values (x) to estimated rubber leaf chlorophyll content (mg/g) (y)
Tabel 2. Persamaan regresi untuk mengkonversi nilai indeks atLEAF CHL PLUS (x) menjadi perkiraan kandungan klorofil daun karet (mg/g) (y)

Variable *Variabel*		Regression *Regresi*	R^2 *R^2*	RMSE *GARK*	CV *KK*
Clones *Klon*	Chlorophyll *Klorofil*				
SP 217	Chlorophyll a	y = 0.0134x - 0.0645	0.9962	0.01394	2.88668
	Chlorophyll b	y = 0.0114x - 0.0738	0.9879	0.02112	5.42562
	Chlorophyll a + b	y = 0.0248x - 0.1379	0.9947	0.03035	3.48022
PB 260	Chlorophyll a	y = 0.0078x + 0.2822	0.9146	0.01814	2.60059
	Chlorophyll b	y = 0.0057x + 0.2445	0.7445	0.02540	4.63788
	Chlorophyll a + b	y = 0.0135x + 0.5252	0.8648	0.04066	3.26771
GT1	Chlorophyll a	y = 0.0120x + 0.1009	0.8184	0.03996	5.90465
	Chlorophyll b	y = 0.0119x - 0.0331	0.9318	0.02259	4.22887
	Chlorophyll a + b	y = 0.0239x + 0.0665	0.8852	0.06068	5.01210
All clones (general)	Chlorophyll a	y = 0.0136x - 0.0257	0.9014	0.05249	8.51884
	Chlorophyll b	y = 0.0111x - 0.0361	0.9076	0.04136	8.47271
	Chlorophyll a + b	y = 0.0247x - 0.0615	0.9087	0.09133	8.27272

R^2: Coefficient of Determination. RMSE: Root Mean Square Error, CV: Coefficient of Variation.
R^2: Koefisien Determinasi, GARK: Galat Akar Rerata Kuadrat, KK: Koefisien Keragaman

The relationship between atLEAF CHL PLUS and SPAD-502 values was linear with significant coefficient of determination (R^2) of 0.9652, 0.9338, and 0.8545, and 0.9497 for SP 217, PB 260, GT1, and all clones, respectively. Regression equation to convert atLEAF CHL PLUS values to SPAD-502 values and to convert SPAD-502 values to atLEAF CHL PLUS values as well as RMSE (root mean square error), and CV (coefficient of variation) are presented in Table 3 and 4, respectively.

Table 3 and 4 show that RMSE and CV of the conversion of both from SPAD-502 values to atLEAF CHL PLUS values and from atLEAF CHL PLUS to SPAD-502 values are relatively small. RMSE and CV indicate how well the regression formula fits the observed data (Lee & Lu, 2010), hence the small RMSE and CV indicated that the estimated data fits the observed data. Therefore, the value resulted from SPAD-502 and atLEAF CHL PLUS were closely correlated, hence both of the devices can substitute each other.

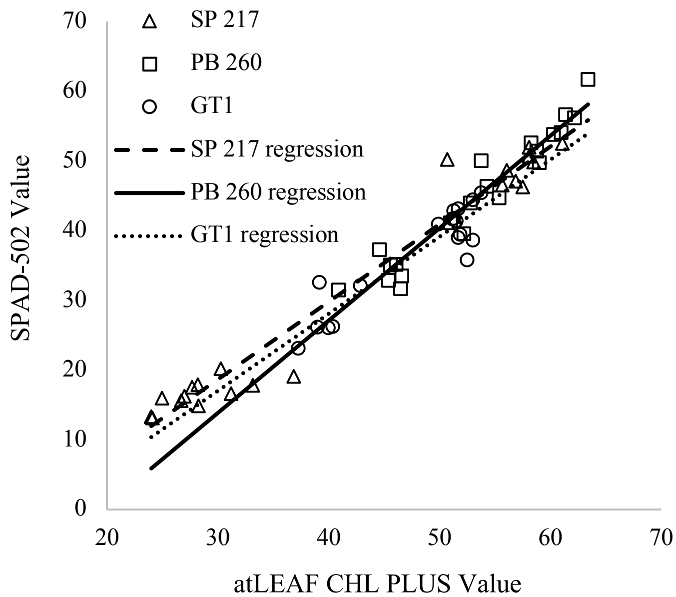

Figure 2. Regression between atLEAF CHL PLUS and SPAD-502 values in three rubber clones
Gambar 2. Regresi antara nilai indeks atLEAF CHL PLUS dan SPAD-502 pada tiga klon karet

Table 3. Regression equation to convert atLEAF CHL PLUS values (x) to SPAD-502 values (y) and descriptive statistics in three rubber clones
Tabel 3. Persamaan regresi untuk mengkonversi nilai indeks atLEAF CHL PLUS (x) menjadi nilai indeks SPAD-502 (y) dan deskripsi statistik pada tiga klon karet

Rubber clone *Klon karet*	Regression equation *Persamaan regresi*	R^2 R^2	RMSE *GARK*	CV *KK*
SP 217	y = 1.1134x - 14.750	0.9652	3.2068	10.4813
PB 260	y = 1.3253x - 25.918	0.9338	2.4937	5.5741
GT1	y = 1.1051x - 16.141	0.8545	2.8263	7.6895
All clones	y = 1.1388x - 16.443	0.9497	3.0015	8.0267

R^2: Coefficient of Determination. RMSE: Root Mean Square Error, CV: Coefficient of Variation.
R^2: Koefisien Determinasi, GARK: Galat Akar Rerata Kuadrat, KK: Koefisien Keragaman

Table 4. Regression equation to convert SPAD-502 values (x) to atLEAF CHL PLUS values (y) and descriptive statistics for rubber clones SP 217, PB 260, and GT1.
Tabel 4. Persamaan regresi untuk mengkonversi nilai indeks SPAD-502 (x) menjadi nilai indeks atLEAF CHL PLUS (y) dan deskripsi statistik untuk tanaman karet klon SP 217, PB 260, dan GT1.

Rubber clone *Klon karet*	Regression equation *Persamaan regresi*	R^2 R^2	RMSE *GARK*	CV *KK*
SP 217	y = 0.8670x + 14.203	0.9652	2.8298	6.9480
PB 260	y = 0.7046x + 21.790	0.9338	1.8184	3.4106
GT1	y = 0.7733x + 19.445	0.8545	2.3642	4.9392
All clones	y = 0.8340x + 16.089	0.9497	2.5686	5.4333

R^2: Coefficient of Determination. RMSE: Root Mean Square Error, CV: Coefficient of Variation.
R^2: Koefisien Determinasi, GARK: Galat Akar Rerata Kuadrat, KK: Koefisien Keragaman

Conclusion

Rubber leaves index values observed by SPAD-502 and atLEAF CHL PLUS were closely correlated, hence both of the devices can substitute each other. In addition, the correlation between atLEAF CHL PLUS and SPAD-502 values with absolute chlorophyll content of clone SP 217, PB 260, GT1, and all clones (general) were significant with a high coefficient of determination (R^2) as well as low RMSE and CV. Therefore, both atLEAF CHL PLUS and SPAD-502 can be used to make fast and non-destructive prediction of chlorophyll content of rubber plant leaf. The equation to convert relative indicator of chlorophyll content value of general rubber leaf to estimated (absolute) total chlorophyll content were $y = 0.0203x + 0.3451$ for SPAD-502 and $y = 0.0247x - 0.0615$ for atLEAF CHL PLUS, where y = estimated chlorophyll content (mg/g) and x = SPAD-502 or atLEAF CHL PLUS index value.

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